Noisy pneumothorax is a benign condition, usually occurring in young healthy males, and is invariably left-sided. The mechanism of production of the sounds is related to a localized collection of air in the pleura being compressed and moved on the medial aspect of the left lung by the forcible action of the ventricles.

In the past the condition has often been reported as "benign spontaneous mediastinal emphysema," but there is considerable room for doubt about this diagnosis as a clinical entity.

Shallow spontaneous pneumothorax presumably occurs as often on the right side, but it is rarely recognized because of the absence of Hamman's sign and lack of proximity of the pain to the praecordium. In a healthy patient with sudden chest pain this diagnosis cannot be excluded without an *x*-ray film taken in full expiration.

ADDENDUM.—Since this paper was accepted we have encountered an example of right-sided shallow apical pneumothorax accompanied by a faint crackling noise down the right border of the sternum. So far as we can ascertain, this is the first recorded example, although Black (1948) did describe the case of a patient with a complete right pneumothorax who experienced a clicking sound while walking. In such a case the presence of a small quantity of fluid may have been responsible for the production of the sounds, and the example is scarcely comparable. Our patient was Case 23 in the above series, who had had a typical left noisy pneumothorax 11 months before. It would appear that in the case of an active heart the right atrial surface may occasionally disturb an air pocket sufficiently to produce at least a faintly audible sound.

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The two-year mass radiography campaign in Scotland, in which 1,844,268 people were examined, disclosed 12,094 new cases of tuberculosis. There was an exceptional public response, but at least one-third of adults in the survey areas failed to co-operate. Evidence suggests that disease among these people is as high as, if not higher than, among those who did co-operate. A report of the campaign, *The Two-year Mass Radiography Campaign in Scotland 1957–58*, (H.M.S.O., price 7s.), makes the following points: nearly two-thirds of the cases were found in Glasgow; nearly four-fifths were found in Glasgow, Edinburgh, Aberdeen, and Dundee; new cases of tuberculosis were mostly among older men and younger women; about two-thirds of all cases were among men; fewer than expected were recalled for full-size radiography; the total number radiographically examined was some 50% higher than expected. A feature of the campaign was the relatively better support given by women in all age groups. The towns in which the male response was the greater were Edinburgh, Dundee, and Aberdeen. The highest response rates were by both men and women belonging to the 15 to 25 and 45 to 59 age groups. The report adds that the trend of tuberculosis will require continued study over a period of years to determine the long-term influence of this campaign.

# MALARIA IN AFRICAN CHILDREN WITH DEFICIENT ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE

BY

**A. C. ALLISON, M.A., D.Phil., B.M.**
*National Institute for Medical Research, London*

AND

**D. F. CLYDE, M.D., D.T.M.&H.**
*Specialist Malariologist, Tanganyika*

It has been recognized for twenty years that some subjects—particularly among populations of African, Asian, or Mediterranean origin—are apt to develop haemolysis when given therapeutic doses of the anti-malarial 8-aminoquinolines (primaquine, pamaquin, etc.), sulphonamides, and certain other drugs. Beutler (1959) and his colleagues showed that sensitivity to primaquine is due to an intrinsic erythrocyte defect. One of the most striking features of the defect is diminished activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD) which catalyses the first and rate-controlling step in the hexose monophosphate shunt metabolic pathway (Carson *et al.*, 1956). It has been suggested that the low G6PD in sensitive subjects may not be the primary defect, but that stromal activation of the enzyme may be at fault (Rimon *et al.*, 1960).

Childs and Zinkham (1959) and others have shown that the defect is inherited as a sex-linked character, with full expression in male hemizygotes (having an X chromosome carrying the mutant gene, together with a normal Y chromosome) and female homozygotes (having two X chromosomes carrying the mutant gene) and partial expression in female heterozygotes (having an X chromosome carrying the mutant gene and a normal X chromosome).

Although the G6PD-deficiency trait is not usually associated with haemolysis, there is no doubt that it is potentially harmful. In Mediterranean countries subjects with the trait develop favism after ingestion of broad beans (see Beutler, 1959), and evidence is accumulating that they suffer from haemolysis when exposed to virus infections (Szeinberg *et al.*, 1960; Marks, 1960). In spite of this hazard, the enzyme-deficiency trait has attained high frequencies in certain areas, including East Africa, where in some tribes it is found in 25% of males (Allison, 1960). As Motulsky (1960) and Allison (1960) have emphasized, the distribution of the enzyme-deficiency trait parallels that of *Plasmodium falciparum* malaria until recent eradication. The question therefore arises whether the trait has become common in malarious regions because carriers are protected to some extent against falciparum malaria, as is the case with the sickle-cell gene (Allison, 1957).

The present investigation was undertaken to test this hypothesis directly by measuring parasite rates and densities in susceptible African children with and without enzyme deficiency, living in a holoendemic area. Young children were selected for analysis, as in an earlier investigation of malaria and the sickle-cell trait (Allison, 1954), because in older children and adults the effects of acquired immunity may be so great as to overshadow inherited resistance to malaria (Allison, 1957).

### Selection of Subjects and Methods of Study

The subjects were 532 African children aged 4 months to 4 years living in the Tanga and Korogwe districts of Tanganyika. They belonged to several tribes, mainly Bondei, but with smaller numbers of Sambaa, Zigua, Digo, and others. In most of the region investigated the rate of transmission is extremely high, as shown by the field study of Davidson and Draper (1953) in a near-by valley. In some parts—for example, villages on the eastern slope of the Usumbara mountains—the transmission rate is much lower.

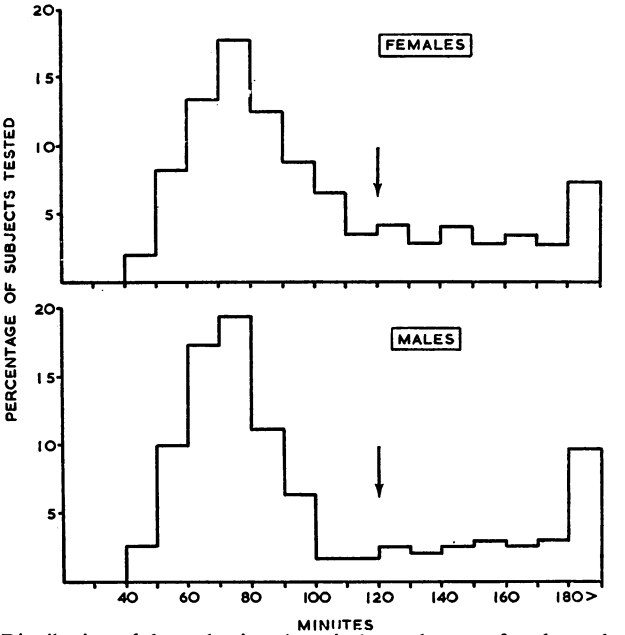
Approximately two-thirds of the tests were made during the course of village surveys at which specimens were taken from a high proportion of children of the appropriate age. Other specimens were obtained at infant welfare and out-patient clinics. The latter may not have been representative of the population at large. However, this form of selection would lead to inclusion of a relatively large proportion of subjects with high malaria parasite counts. Such a bias would be favourable for showing whether the enzyme deficiency imposes any limit on malaria-parasite densities, and there is no reason to suppose that it might artificially produce a difference between subjects with normal and abnormal enzymes if none exists in the general population. Subjects known to have received recent antimalarial therapy were excluded.

A free flow of blood was obtained from heelpricks in infants and fingerpricks in ambulant children ; 0.04 ml. of blood was placed in tubes with acid-citrate-dextrose for enzyme assay, and a thick blood film was taken. Usually a drop of blood was mixed with a freshly prepared isotonic solution of sodium metabisulphite for a sickling test. The majority of children were then given a single dose of 150 mg. of base chloroquine diphosphate. Seven weeks to six months later thick blood films were taken on as many subjects as possible. A higher proportion of the follow-up cases examined up to two months after treatment were found to be parasite-free than in untreated groups, a result which was almost certainly due to the late effects of treatment. It would have been difficult to exclude these subjects without discrimination, so that they have been included in the whole series. A small number of subjects were examined a third time. The total number of tests was 876.

#### Enzyme Assay

All tests were carried out within 48 hours of collecting blood, which was refrigerated overnight. Motulsky's (1960) method of estimating glucose-6-phosphate dehydrogenase activity of haemolysates was used. To compensate for the anaemia which was common in the subjects tested, the red cells preserved in acid-citrate-dextrose were lysed in distilled water and the haemolysate was diluted until the haemoglobin concentration was equivalent to 0.02 ml. of blood with a haemoglobin of 12 g./100 ml. Then 1 ml. of the haemolysate was taken and mixed with buffered glucose-6-phosphate, triphosphopyridine nucleotide, and brilliant cresyl blue as described by Motulsky. The final decoloration was quite rapid and no difficulty was experienced in recording an end-point. The decoloration times for males and females are shown in the Chart. For males there is a fairly clear antimode distinguishing enzyme-deficient hemizygotes from normals. For females the point of distinction is less obvious and the time taken as the limit of normality certainly includes a proportion of heterozygotes, but probably few, if any, abnormal homozygotes. This can be deduced from the population genetics of the condition.

The frequency of the G6PD gene (p) in the whole population is the same as the hemizygote frequency in the male population, in this case 0.25. The expected frequency of heterozygotes plus homozygotes in the female population is $2p(1-p)+p^2$—that is, 0.44—whereas the recorded frequency of enzyme-deficient females is only 0.27. However, since the heterozygotes showing low enzyme activities will be detected, this method of recording provides a valid basis for comparison of malaria in enzyme-deficient and normal subjects. A further point deserving consideration is the relatively high G6PD activity in young cells (Marks *et al.*, 1958). If they had a considerable reticulocytosis, G6PD-deficient subjects might temporarily show normal enzyme activity, and if this happened in subjects with the highest malaria parasite counts there could be a bias showing apparent protection of enzyme deficiency against malaria where none, in fact, exists. However, for two reasons this seems unlikely. In the first place, the shower of reticulocytes follows the peak of parasitaemia and is not coincident with it ; and, second, the reticulocytosis from malaria is seldom great enough to produce misclassification of enzyme-deficient subjects. Malaria parasite counts were made against leucocytes on thick films. The parasite counts and the sickling and enzyme tests were carried out independently by the two of us and the results later exchanged.



Distribution of dye-reduction times in haemolysates of males and females. Arrows mark the time (120 minutes) taken as the upper limit of normality.

### Results

*Malaria and Enzyme Deficiency.*—The incidence of *P. falciparum* malaria parasites in normal and enzyme-deficient children is shown in Table I. It will be seen that in both sexes the parasite rates are lower in enzyme-deficient subjects. In males the difference is statistically significant at the 5% level, but this is not so for females. The parasite densities in Table II again show evidence of protection by enzyme deficiency, since the proportions of subjects with high parasite densities are lower in both the male and the female groups with G6PD deficiency than in the corresponding groups with normal enzymes.

TABLE I.—*P. Falciparum Malaria Parasite Rates in Children with Normal and Deficient G6PD*

| | No. Tested | Showing Parasites | | Significance |
|---|---|---|---|---|
| | | No. | % | |
| Males with normal enzymes | 319 | 210 | 65·8 | $\chi^2=4·28$ for 1 d.f. $P<0·05$ |
| ,, ,, G6PD deficiency | 105 | 58 | 55·2 | |
| Females with normal enzymes | 331 | 221 | 67·6 | $\chi^2=1·91$ for 1 d.f. $P>0·10$ |
| ,, ,, G6PD deficiency | 121 | 73 | 60·3 | |
| Total | 876 | 562 | 64·2 | |

TABLE II.—*Distributions of P. Falciparum Densities in Infected Children*

| Parasite Counts/μl. Blood | 1–99 | 100–999 | 1,000–9,999 | 10,000+ |
|---|---|---|---|---|
| Males with normal enzymes | 21 (6·6%) | 77 (24·1%) | 71 (22·3%) | 41 (12·9%) |
| Males with G6PD deficiency | 7 (6·7%) | 26 (24·7%) | 19 (18·1%) | 6 (5·7%) |
| Females with normal enzymes | 17 (5·1%) | 72 (21·8%) | 78 (23·6%) | 54 (16·3%) |
| Females with G6PD deficiency | 7 (5·8%) | 28 (23·1%) | 27 (22·3%) | 11 (9·1%) |

A comparison can be made in both sexes of subjects with parasite counts above and below 1,000/μl., since this is the level above which mortality from *P. falciparum* malaria is known to occur (Field, 1949). In the males the difference is statistically significant ($\chi^2$, with Yates's correction for continuity=4.11 for 1 degree of freedom, $P<0.05$). In the females the difference approaches significance at the 5% level, but does not attain it ($\chi^2=3.08$ for 1 d.f. $0.1>P>0.05$).

*Sickling and Malaria.*—For purposes of comparison with enzyme deficiency in the same population, the *P. falciparum* parasite rates and densities are shown in Tables III and IV. Again the parasite rate is significantly lower in the group with the sickle-cell trait, and there is a significantly lower proportion of sickling subjects with counts above 1,000/μl. ($\chi^2=4.85$ for 1 d.f. $P<0.05$). This confirms a substantial body of evidence that in the age-group under consideration sickling affords considerable protection against *P. falciparum* malaria. The degree of protection is of the same order as that recorded in other comparable groups—for example, from Kampala (Raper, 1956) and Léopoldville (Vandepitte and Delaisse, 1957). The protection by sickling in our group appears to be of the same order as that in males with enzyme deficiency, and greater than that in females with enzyme deficiency. The small number of subjects having both sickling and enzyme deficiency do not appear to show greater protection than that provided by either trait alone.

TABLE III.—*P. Falciparum Malaria Parasite Rates in Children Tested for Sickling*

| | No. Tested | Showing Parasites | | Significance |
|---|---|---|---|---|
| | | No. | % | |
| Children without s.c.t. | 407 | 272 | 66·8 | $\chi^2=5·09$ for 1 d.f. $P<0·05$ |
| ,, with ,, | 136 | 77 | 56·6 | |
| ,, ,, ,, and G6PD deficiency | 38 | 22 | 57·9 | |

TABLE IV.—*Distribution of P. Falciparum Parasite Densities in Infected Children Tested for Sickling*

| Parasite Counts/μl. Blood | 1–99 | 100–999 | 1,000–9,999 | 10,000+ |
|---|---|---|---|---|
| Children without s.c.t. | 27 (6·6%) | 93 (22·9%) | 98 (24·1%) | 54 (13·3%) |
| ,, with ,, | 10 (7·4%) | 33 (24·3%) | 26 (19·1%) | 10 (7·4%) |
| ,, ,, ,, and G6PD deficiency | 3 (7·9%) | 9 (23·7%) | 6 (15·8%) | 4 (10·5%) |

*Enzyme Deficiency and Malaria in the Gambia.*—Through the courtesy of Dr. I. A. McGregor, erythrocyte G6PD measurements have been made on 31 Gambian children who had been examined daily for the first two years of life for clinical signs of malaria and number of parasites. Three enzyme-deficient males were included in the sample. Although these children had occasional high parasite counts, the peaks of parasitaemia were briefer than in other children; the only two subjects in the whole group who were judged clinically not to be in danger from malaria throughout the two-year period, and received no antimalarial treatment, were enzyme-deficient. Although these numbers are small, they represent a unique sample that was carefully studied, and the results are consistent with the malaria-protection hypothesis.

## Discussion

The present study has provided substantial evidence that G6PD deficiency in young African children affords a considerable degree of protection against *P. falciparum* malaria. This is manifested by decreased parasite rates and counts in enzyme-deficient subjects, which is most pronounced in hemizygous males but is probably also present in heterozygous females. In a group of older children from the Belgian Congo, Motulsky (1960) found only slight and insignificant protection against malaria in enzyme-deficient subjects. This situation is comparable to that manifested by the sickle-cell trait, in which resistance is rarely demonstrable in children over 5 years of age with considerable acquired immunity (Allison, 1957).

Poor growth of malaria parasites in G6PD-deficient cells would be expected. Plasmodia require reduced glutathione for growth *in vitro* (McGhee and Trager, 1950) and about half of the glutathione of the red cell contributes to the cysteine requirement of the parasites (Fulton and Grant, 1956). Enzyme-deficient cells have a subnormal concentration of glutathione, which in a variety of conditions is depleted to very low levels (Beutler, 1959). Moreover, there is evidence that malaria parasites use the hexose-monophosphate-shunt pathway in red cells (Geiman, 1951), and the rate of metabolism by this pathway is diminished in enzyme-deficient cells. Hence for at least two reasons enzyme-deficient cells would be unlikely to support maximal growth of malaria parasites.

As a result of the present investigation a plausible interpretation of the population genetics of G6PD deficiency can be offered. The female heterozygote is resistant to malaria and is therefore at a selective advantage, while the male hemizygote, being liable to haemolysis, may actually be at a net disadvantage. Heterozygous advantage is the simplest way in which genetic equilibrium at a sex-linked locus can be maintained (Mandel, 1959). If the abnormal homozygote and hemizygote were at a net advantage, the enzyme-deficiency gene might have replaced the normal allele in some populations, whereas the frequency of the former is always less than 0.5. From considerations of population genetics, then, it seems likely that in Africa enzyme deficiency is associated with haemolysis.

The factor precipitating the haemolysis under natural conditions is unknown. In most of Africa the broad bean is not eaten, but the reports of Szeinberg *et al.* (1960) and Marks (1960) that certain virus infections precipitate haemolysis in susceptible subjects may well be relevant. Africans suffer from a great variety of

virus diseases, including many that are arthropod-borne, in which high concentrations of virus are attained in the blood-stream. It seems possible that some epidemics of jaundice not due to yellow fever—for example, that described by Hahn and Bugher (1953)—may have occurred as a result of haemolysis in enzyme-deficient subjects. The observations that some patients had dark urine, and that males were more severely affected than females, are consistent with this interpretation. A further search for haemolysis associated with virus infections should be made.

This discussion has been concerned with some of the selective agents likely to be operating under natural conditions. They have left in African and other populations a genetic legacy that is also a medical hazard, since G6PD-deficient subjects are liable to haemolysis when exposed to a wide range of drugs in therapeutic concentrations. These include the antimalarial 8-aminoquinolines and sulphonamides (Beutler, 1959), and also nitrofurazone, which is used in trypanosomiasis (Robertson, 1960). Indeed, it appears likely that many of the alleged cases of blackwater fever reported in immune Africans are due to severe haemolytic episodes in G6PD-deficient subjects; several such cases in Nigeria have been found by Gilles (private communication) to have enzyme deficiency.

**Summary**

*P. falciparum* malaria parasite rates and densities were found to be significantly lower in young African children with erythrocyte glucose-6-phosphate dehydrogenase deficiency than in children with normal enzymes. Reasons are given in the discussion why this might be so. This fact helps to explain why the enzyme-deficiency trait remains common in malarious areas even though it predisposes carriers to haemolysis under certain conditions.

The enzyme tests were carried out in laboratory space provided through the courtesy of the late Dr. D. B. Wilson, at Amani, Tanganyika. The visit of one of us (A. C. A.) to East Africa was made possible by a grant from the Colonial Development and Welfare Fund, recommended by the Colonial Medical Research Committee (now the Tropical Medicine Research Board).

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# INCREASE IN HAEMOGLOBIN $A_2$ APPEARING AFTER HOMOGRAFT OF FOETAL HAEMOPOIETIC TISSUE

BY

**J. M. BRIDGES, M.D.**

**D. W. NEILL, M.Sc., F.R.I.C.**
*Royal Victoria Hospital, Belfast*

AND

**H. LEHMANN, M.D., Sc.D., M.R.C.P., F.R.I.C.**
*St. Bartholomew's Hospital, London*

The use of chemotherapy for the treatment of cancer has increased greatly in recent years, but as yet no drug has been produced which has a specific action on the malignant process. A factor which often limits the use of these drugs in effective doses is the vulnerability of the bone-marrow to toxic damage by them. If this toxic damage to the haemopoietic system could be efficiently treated then larger doses of the drugs could be employed. The possibility of the use of infusions of haemopoietic cells in the management of this condition has recently aroused much interest. The effectiveness of such treatment will depend on whether the transfused haemopoietic cells mature and are released into the host's circulation.

Two methods are commonly used to determine if cells derived from the transfused material are circulating in the host peripheral blood. The first depends on obtaining the transfused material from a donor of the opposite sex; then, by examining the circulating neutrophils for nuclear appendages, they can be "sexed" and so it can be determined if they arose from host or donor haemopoietic tissue. The second is dependent on obtaining the transfused material from a donor of different blood group from the host; then, by differential agglutination studies of the host peripheral blood, the presence or absence of red cells of the donor group can be demonstrated. Both these methods have technical limitations and also restrict the choice of donor material.

Suspensions of foetal liver contain large numbers of haemopoietic cells. If such foetal material is transfused and the erythropoietic elements survive and mature, then it seems possible that foetal haemoglobin may be produced and circulate in the host's blood. If this were so, then the detection of this haemoglobin would serve as an indication of the maturation of the transfused erythropoietic cells.

The present paper describes our experience in examining the blood of patients for the presence of unusual haemoglobins after use of foetal haemopoietic cell infusions for severe toxic marrow failure.

Eight patients suffering from severe marrow failure subsequent to intensive chemotherapy for various malignant conditions were given transfusions of foetal liver suspension.* The method of collection and storage of this material has been described by Kay and Constandoulakis (1959a). At the time of transfusion all patients were severely ill and showed anaemia, leucopenia, and thrombocytopenia, indicating toxic depression of all facets of marrow function.

In five patients death occurred within nine days of transfusion with no evidence of recovery of haemopoietic function: in none of these patients was any

*The foetal liver suspension was kindly supplied to us by Dr. H. E. M. Kay, of the Royal Marsden Hospital, London.